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**Photoactivatable two-stage protective groups for the
synthesis of biopolymers**

Description

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The present invention relates to a process for synthesizing biopolymers by stepwise assembly from protected synthesis building blocks which carry two-stage protective groups. The two-stage protective groups are activated by a first illumination step and eliminated by a subsequent chemical treatment step. Photoactivatable components which considerably speed up the activation process via intramolecular triplet sensitizers or/and have fluorescence properties are used. The two-stage protective groups can be used in particular within the framework of quality control.

The technology of light-controlled synthesis of biopolymers using photolabile protective groups opens up the possibility of producing biochips in situ by synthesis from monomeric and oligomeric building blocks. Biochips have gained a very considerable importance for research and diagnosis since they permit rapid and highly parallel processing of complex biological problems. However, chips of the highest quality are required for this, so that there is a great interest in novel and more efficient synthetic methods.

Photolabile nucleoside derivatives are used in the light-controlled synthesis of nucleic acid chips. In this connection, the assembly of the chain of nucleic acid fragments normally takes place using phosphoramidite synthons. The building blocks each carry a temporary photoprotective group which can be removed by incident light. The principle of the synthesis provides for a cyclic sequence of condensation and deprotection steps (by light). The efficiency with which such a light-controlled synthesis can take place is determined essentially by the photolabile protective groups used,

in particular by the efficiency with which they can be removed in the irradiation step. The photoprotective groups used to date for light-controlled synthesis are normally the protective groups NVOC (S.P.A. Fodor et al., Science 251 (1991), 767 ff.), MeNPOC (A.C. Pease et al., Proc. Natl. Acad. Sci. 91 (1994), 5022 ff.), DMBOC (M.C. Pirrung, J. Chem. 60 (1995), 1116 ff.) and NPPOC (A. Hassan et al., Tetrahedron 53 (1997), 4247 ff.). Further known photolabile protective groups in nucleoside and nucleotide chemistry are o-nitrobenzyl groups and their derivatives (cf., for example, Pillai, Org. Photochem. 9 (1987), 225; Walker et al., J. Am. Chem. Soc. 110 (1988), 7170). A further photolabile protective group which has been proposed is the 2-(o-nitrophenyl)ethyl group (Pfleiderer et al., in: "Biosphosphates and their Analogues - Synthesis, Structure, Metabolism and Activity", ELSEVIER Science Publishers B.V. Amsterdam (1987), 133 ff.) and derivatives thereof (WO 97/44345 and WO 96/18634).

The photolabile protective groups currently used for light-controlled synthesis of nucleic acids (e.g. NVOC, MeNPOC, NPPOC) are generally distinguished by a comparatively low absorption coefficient at the wavelength of the incident light. Irradiation of photolabile nucleoside derivatives normally takes place with high pressure Hg lamps at a wavelength of 365 nm. The result of the low absorption coefficient of the photolabile protective group used at this wavelength is that only a very small proportion of the incident light can be utilized for excitation of the molecules. In addition, the photolabile protective groups used are mostly colorless derivatives. The result of this in turn is that it is not possible during the synthesis to detect by simple spectroscopic methods whether the photolabile protective group is still present on the nucleoside derivative or has already been partly or completely abstracted by the input of light. The abstraction process can thus be followed only with

difficulty or not at all.

DE 101 32 925.6 and PCT/EP02/07389 propose the use of two-stage protective groups, where the two-stage
5 protective groups are activated by an illumination step and eliminated by a subsequent chemical treatment step. The two-stage protective groups are preferably trityl derivatives which are coupled to a photoactivatable protective group. The trityl derivatives may also
10 comprise fluorescent groups in addition.

In order to eliminate the disadvantages of the previously published prior art, according to the present invention specific photoactivatable groups are
15 linked to a second component whose elimination conditions are orthogonal to those of the photo-activatable groups, and whose removal leads to exposure of the actual protective group which can be eliminated by chemical means, e.g. acid catalysis. The protective
20 group eliminated by chemical means, which is preferably colored or/and fluorescent, can be employed for quality control during the synthesis of biopolymers.

The present invention provides a novel protective group
25 with which the activation step is induced by light and the actual deprotection step at the reaction site takes place by chemical means, e.g. acid catalysis (fig. 1). This novel protective group, and molecules carrying this protective group, can be employed for the
30 synthesis of biopolymers.

One aspect of the invention is thus a process for the synthesis of biopolymers by stepwise assembly from
35 synthesis building blocks which carry protective groups, with use of at least one synthesis building block which carries a two-stage protective group which is activated by an illumination step and is eliminated by a subsequent chemical treatment step, using as photoactivatable group a triplet-sensitized photo-

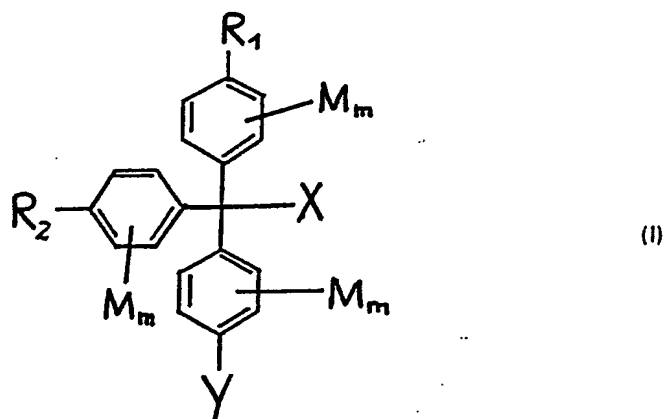
activatable group, a labeled, e.g. fluorescent photo-
activatable group or/and a labeled, e.g. fluorescent
and triplet-sensitized photoactivatable group. The
illumination step preferably comprises the elimination
5 of a first photolabile component of the protective
group, leaving behind a second component of the
protective group which is essentially stable to the
conditions prevailing on elimination of the first
component and which can subsequently be eliminated by a
10 chemical treatment step. The chemical treatment step
preferably comprises a treatment with base, a treatment
with acid, an oxidation, a reduction or/and catalytic,
e.g. an enzymatic, reaction. The chemical treatment
step particularly preferably comprises an acid
15 treatment.

In a particularly preferred embodiment of the inven-
tion, a derivatized trityl group is used as two-stage
protective group. Trityl groups are notable for their
20 excellent ease of elimination, in particular by treat-
ment with acid. The two-stage trityl protective groups
of the invention are, by contrast, not acid-labile but
are converted into an acid-labile form only after
activation and elimination of one or more photolabile
25 components.

Triplet-sensitized photoactivatable groups and labeled,
e.g. fluorescent, and triplet-sensitized photo-
activatable groups on the one hand have a high molar
30 extinction coefficient at the incident wavelength in
order to contribute to a significant increase in the
population in the triplet state, and on the other hand
are able to stabilize a tertiary free radical in the
aci-nitro form via I or M effects. This leads to an
35 increase in the overall quantum yield of the activation
step. Labeled photoactivatable groups (without triplet
sensitization) show merely a high molar extinction
coefficient at the incident wavelength, but have no
direct effect on the activation process. All said types

of photoactivatable groups are particularly suitable in quality control, for example in fluidic microprocessors as described for example in WO 00/13018.

- 5 Particular preference is therefore given to a synthesis building block which has a two-stage protective group and which has the general formula (I):



- 10 where R_1 and R_2 are each independently selected from hydrogen, $(L)-R_3$, $O-(L)-R_3$, $N(R_3)_2$, NH_2 and M , R_3 is a C_1-C_8 alkyl group, a C_2-C_8 -alkenyl group, a C_2-C_8 -alkynyl group, a C_6-C_{25} aryl group or/and a C_5-C_{25} -heteroaryl group, each of which may optionally have one or more substituents, L is a linker group which is optionally present, which is for example $-(CH_2)_n-$, $-(CH_2)_n-COO-$, $-(CH_2)_n-CONH-$ or $-(CH_2)_n-SO_2O-$, $-(CH_2)_n-O-$, $-(CH_2)_n-S-$ or $-(CH_2)_n-NH-$, and n is an integer from 0 to 20, M is in each case independently a label optionally linked via a linker group L (as defined above), and m is in each case independently an integer from 0 to 4, preferably 0, 1 or 2, X is the synthesis building block, Y is in each case independently a photoactivatable protective group as indicated above, Z is an amino protective group, and where R_1 or/and R_2 may optionally be replaced by Y .

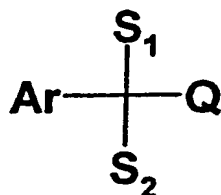
The alkyl, alkenyl and alkynyl groups may be linear or

cyclic, straight-chain or branched. The aryl or hetero-
aryl groups, e.g. N-, O- or/and S-heteroaryl groups,
may be mono- or polycyclic. Examples of substituents of
alkyl, alkenyl, alkynyl, aryl and heteroaryl groups are
5 halogen, e.g. F, Cl, Br, I, OH, SH, -O-, -S-, -S(O)₂-,
NO₂, CN, COOH, CO-C₁-C₈-alkyl, COO-C₁-C₈-alkyl, OCO-C₁-
C₈-alkyl, CONH-C₁-C₈-alkyl, CON-(C₁-C₈)-alkyl)₂, C₁-C₈-
alkyl, C₂-C₈-alkenyl, C₂-C₈-alkynyl, C₁-C₈-alkoxy, -S-C₁-
C₈-alkyl, di(C₁-C₈-alkyl)amino and NHZ, where the alkyl,
10 alkenyl, alkynyl and alkoxycarbonyl groups may in turn
be substituted by halogen. Preferred meanings for R₁
and R₂ are hydrogen, dialkylamine, e.g. N,N-dimethyl,
O-methyl, OCOO-methyl or a protective amino group, e.g.
an amino group converted into an amide function with a
15 suitable carboxylic acid. The number of carbon atoms in
the radicals R₁ and R₂ of the compound is preferably
restricted to 25 in each case.

The invention also encompasses compounds which carry a
20 plurality of photoactivatable groups, in particular
compounds of the formula (I) in which at least one of
R₁ or R₂ is replaced by a photoactivatable protective
group. It is preferred for 1 to 3 photoactivatable
protective groups to be present. It is additionally
25 possible for one or more labeling groups to be present
and to be linked to the photoactivatable component
or/and to the chemically active component. Thus, one or
more labeling groups in the compounds (I) may be
present at the o or/and m positions of the phenyl rings
30 in the trityl system.

It is possible by varying the radicals R₁ and R₂ and
substituting one or both radicals by photoactivatable
protective groups to adapt the acid lability to the
35 desired requirements.

In a preferred embodiment, labeled photoactivatable
groups of the formula (II) are used:



(III)

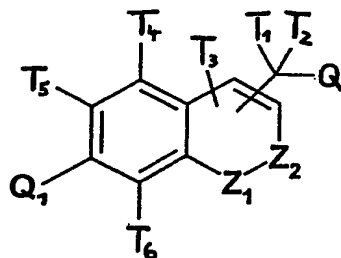
in which Ar is a fused polycyclic, preferably tetra-,
penta- or hexacyclic, fluorescent aryl or heteroaryl,
5 S₁ and S₂ are each independently selected from hydrogen,
a C₁-C₈-alkyl group, a C₂-C₈-alkenyl group, a C₂-C₈-
alkynyl group, a C₆-C₂₅-aryl or/and a C₅-C₂₅-heteroaryl
group, each of which may optionally have substituents,
and Q is a group for linking the photolabile component
10 to the component which can be eliminated chemically.
The number of carbon atoms in the radicals Ar, S₁ and S₂
of the compound (II) is preferably restricted to 25 in
each case.

15 Examples of suitable fluorescent aryl radicals are
benzo[b]fluoranthrene, fluoranthrene, 9,10-diphenyl-
anthracene, acenaphthylene or pyrene.

Substituents of the groups Ar, S₁ and S₂ are as defined
20 above for the compounds of the formula (I). Q is
preferably SO₂, OCO, OCS or CS₂.

Particular preference is given to compounds (II) in
which S₁ and S₂ are H, e.g. the compound PyMOC (as
25 indicated in US patent 6,147,205).

In a further preferred embodiment there is use of a
photoactivatable group of the general formula (III)

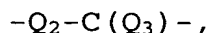


(III)

in which T₁, T₂, T₃, T₄, T₅ and T₆ are each independently
 selected from hydrogen, C₁-C₈-alkyl, C₂-C₈-alkenyl, C₂-
 5 C₈-alkynyl, C₁-C₈-alkoxy, C₂-C₈-alkoxycarbonyl, C₆-C₂₅-
 aryl or aryloxy or/and C₅-C₂₅-heteroaryl or
 heteroaryloxy, each of which may optionally have
 substituents, and T₁ or/and T₂ may additionally be
 trialkylsilyl, and one of T₃ and T₄ may be NO₂, with the
 10 proviso that the other is then H, Q₁ is hydrogen,
 optionally substituted C₁-C₄-alkoxy or di(C₁-C₄-alkyl)-
 amino, Z₁ and Z₂ together are -OC(O)-, -NT₇C(O)- or
 -CT₈=CT₉, where T₈ and T₉ are defined as T₃ - T₆, and T₉
 may additionally be NO₂, and adjacent groups, e.g. T₈
 15 and T₉, may form a 5- or 6-membered carbocyclic or
 heterocyclic, saturated or unsaturated ring, and Q is a
 group for linking the photolabile component to the
 component which can be eliminated chemically. The
 number of carbon atoms in the radicals T₁-T₉ of compound
 20 (III) is preferably restricted to 25 in each case.

The possible substituents of the respective groups are
 in this case as defined above for the compounds of the
 formula (I). Q is preferably a group of the general
 formula:

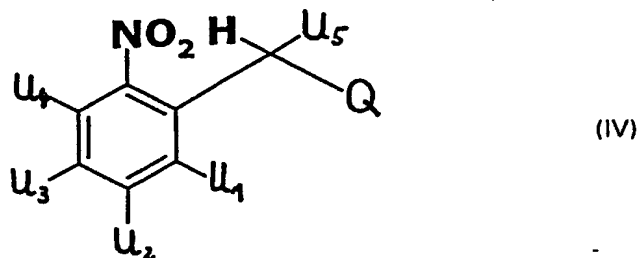
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where Q₂ is -O-, -S-, -CH₂O- or -CH₂S-, and Q₃ is =O or
 =S. Examples of suitable compounds of the formula (III)
 30 are described for example in WO 02/20150.

In yet a further preferred embodiment there is use of

labeled photoactivatable groups of the general formula (IV):



5 in which U_1 , U_2 , U_4 and U_5 are each independently selected from hydrogen, halogen, NO_2 , U_6 , $(L)-U_6$, $O-(L)-U_6$, $N(U_6)_2$ and NHZ , U_6 is C_1-C_8 -alkyl, C_2-C_8 -alkenyl, C_2-C_8 -alkynyl, C_6-C_{25} -aryl or C_5-C_{25} -heteroaryl, each of
 10 which may optionally have substituents, L is a linker group which is optionally present, e.g. as defined for the compounds (I), U_3 is a labeling group optionally linked via a linker group, e.g. as defined for the compound (I), e.g. a fluorescent group, and Q is a
 15 group for linking the photolabile component to the component which can be eliminated chemically. The number of carbon atoms in the radicals $U_1 - U_5$ is preferably restricted to 25 in each case. Adjacent radicals may optionally form a 5- or 6-membered carbo-
 20 cyclic or heterocyclic, saturated or unsaturated ring.

The definition of the possible substituents on the radicals U_1 , U_2 , U_4 and U_5 is as described for the compounds (I). Q is preferably SO_2 , OCO , OCS , CS_2 ,
 25 CH_2SO_4 , CH_2OCO , CH_2OCS , CH_2CS_2 etc. The radical U_3 preferably has the structure $-O-L-NHCOM$ where L is a linker having a chain length of preferably 1-10 atoms, e.g. C atoms, and optionally heteroatoms such as O, S or N, and M is a labeling group, e.g. a fluorescent
 30 group such as, for example, a pyrene or coumarin group.

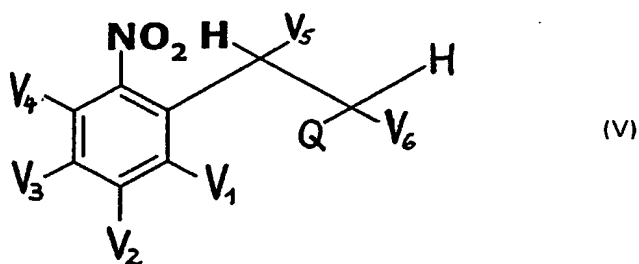
The compounds of this type are based on conventional O-

nitrobenzyl groups such as, for example, NPPOC, NVOC, MeNPOC, into which a fluorophore has additionally been introduced. The resulting photoactivatable molecule has a label but not a triplet sensitization.

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In yet a further preferred embodiment there is use of photoactivatable groups of the formula (V) which are preferably triplet-sensitized and optionally labeled groups:

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in which V_1 , V_2 , V_3 , V_4 , V_5 and V_6 are each independently selected from hydrogen, halogen, NO_2 , V_7 , (L)- V_7 , O-(L)- V_7 , $\text{N}(V_7)_2$, NH_2 and M, where V_7 is C_1 - C_8 -alkyl, C_2 - C_8 -alkenyl, C_2 - C_8 -alkynyl, C_6 - C_{25} -aryl or C_5 - C_{25} -heteroaryl, each of which may optionally have substituents, L is a linker group which is optionally present, e.g. as defined for the compounds (I), V_5 and V_6 may additionally be trialkylsilyl, M is a label optionally linked via a linker group, and Q is a group for linking the photolabile component to the component which can be eliminated chemically. The number of carbon atoms in the radicals V_1 - V_6 is preferably restricted to 25 in each case. Adjacent radicals may optionally form a 5- or 6-membered carbocyclic or heterocyclic, saturated or unsaturated ring.

The radical V_5 is particularly preferably an aryl, aryloxy, heteroaryl or heteroaryloxy group which may be unsubstituted or may have up to three substituents (as defined above). Particular preference is given to polycyclic aryl, aryloxy, heteroaryl or heteroaryloxy

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groups which show triplet sensitization and which optionally may have an intrinsic fluorescence, especially if they comprise four or more fused rings, e.g. pyrenes, benzo[b]fluoranthrenes, fluoranthrenes, 5 9,10-diphenylanthracenes, acenaphthylenes or corresponding oxy derivatives etc.

The invention also includes compounds which carry a plurality of labels which are detectable independently 10 of one another. Examples of suitable labels are fluorescent groups, luminescent groups, electrically detectable groups, e.g. ferrocenes, colored groups, radioactive groups, groups detectable by NMR etc. The labels preferably comprise at least one fluorescent 15 group, which may be combined with another, independently detectable fluorescent group or another type of label as mentioned above. It is preferred for one label to be linked to the photolabile component of the protective group and for the other label to be 20 linked to the component which can be eliminated chemically, so that selective elimination of the photolabile component can be detected by loss of the first label but retention of the second label, and elimination of the chemical component can be detected 25 by loss of the second label. For example, the invention includes compounds (I) which carry a plurality of fluorescent groups, e.g. compounds in which Y is a fluorescent photoprotective group or/and R₃ and Z are fluorescent groups on the trityl framework (R. Ramage, 30 F.O. Wahl, Tetrahedron Lett., 34 (1993), 7133) or molecules in which the fluorescence has been introduced by substitution on the trityl framework (J.L. Fourrey et al., Tetrahedron Lett., 28 (1987), 5157).

35 In a preferred embodiment, the labeling group on the component which can be eliminated chemically is a fluorescent group, e.g. a coumarin or pyrene group, which is coupled via a linker group, e.g. a group as defined above, to the basic trityl framework, e.g. in

p, o or/and m position of the phenyl rings in the trityl system.

These labeling groups can be employed for quality control of biochips. This can take place for example in biochip supports as described in WO 00/13018. On use of fluorescent labeling groups care must be taken that the excitation and emission wavelengths do not interfere with the photoinduced activation.

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The process of the invention is employed for the synthesis of biopolymers, with the biopolymer to be synthesized being assembled stepwise from a plurality of synthesis building blocks. The process is particularly preferably employed for the synthesis of nucleic acids, e.g. DNA or RNA. However, it should be noted that the process is also suitable for the synthesis of other biopolymers such as, for example, peptides, peptide-nucleic acids (PNAs) or saccharides. The synthesis building block may be a monomeric building block, e.g. a nucleoside derivative or a peptide derivative, or else an oligomeric building block, e.g. a dimer or trimer, i.e. for example a di- or trinucleoside derivative or a di- or tripeptide derivative. The synthesis building block is particularly preferably a phosphoramidite building block. It is, however, also possible to use other nucleotide synthesis building blocks, e.g. phosphate or phosphonate building blocks. A further possibility is also to employ linker or spacer building blocks, e.g. as phosphoramidites, as synthesis building blocks. Particularly preferred linkers or spacers as carriers of two-stage protective groups are described in DE 100 41 539.3.

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The synthesis building blocks of the invention carrying a two-stage protective group generally have more strongly lipophilic properties than the synthesis building blocks used to date in the prior art. The

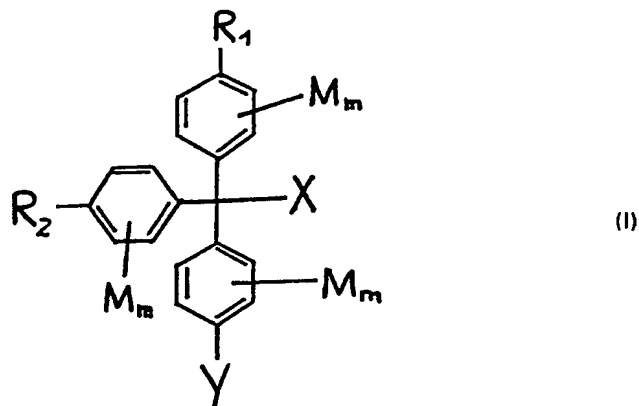
solubility of the synthesis building blocks, especially of the phosphoramidite synthons, in organic solvents is increased through this lipophilicity. The more homogeneous reaction management made possible thereby
5 leads to a higher coupling efficiency compared with the pure photolabile phosphoramidite synthons. Elimination of the colored trityl cation of the photoprotective groups of the invention, which has a considerably higher absorption coefficient than the elimination
10 products in other photodeprotection processes, also opens up the possibility of direct online process monitoring. This leads to an improvement in the quality control of biochips.

15 The trityl group of the photoprotective groups of the invention additionally makes selective functionalization of the 5'-hydroxy function possible. This leads to an enormous reduction in costs, because separation of the 3'-5' isomers is dispensed with.

20 Particular preference is therefore given according to the present invention to phosphoramidite building blocks which carry the two-stage protective group on the 5'-O atom of the sugar, in particular of the ribose
25 or of the deoxyribose.

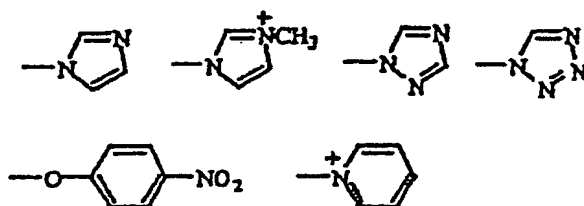
The synthesis of the biopolymers can be carried out in a conventional way, for example on a solid phase. It is particularly preferred for a plurality of biopolymers
30 carrying a different sequence of synthesis building blocks to be generated in situ in the form of an array on a single support.

Yet a further aspect of the invention are compounds of
35 the general formula (I)



where R_1 , Y , M and m are as defined above, and X is a synthesis building block for synthesizing biopolymers or a leaving group, and where R_1 or/and R_2 may optionally be replaced by Y .

If X is a leaving group, it is a group which can be eliminated on reaction of the compound (I) with another compound. X is preferably a leaving group which can be eliminated by reaction with a nucleophile, optionally in the presence of an auxiliary base such as pyridine. Preferred examples of X are: Cl , Br , I , tosylate, mesylate, trifluorosulfonate etc.



The diagrammatic representation of the protective group concept of the invention is shown in **Figure 1**. The synthesis building block (A) carries a two-stage protective group (B-C). In a first illumination step, the photolabile portion (B) of the protective group is eliminated. The chemically labile component (C) of the

protective group is eliminated in a second chemical treatment step, e.g. by addition of acid, so that the synthesis building block (A) is present in active form.

5 **Figures 2 and 3** show exemplary substances from a preferred class of two-stage protective groups of the invention. They are based on the acid-labile trityl group, but comprise in the p position of one phenyl radical a photolabile triplet-sensitized component (V)
10 which reduces or completely blocks the acid sensitivity of the trityl group. The photolabile component in Figure 3 shows intrinsic fluorescence. The protective group is converted into an acid-labile form by illumination and elimination of the photolabile
15 component and can subsequently be eliminated in the presence of acid to liberate the unprotected synthesis building block.

Figure 4 shows a further exemplary substance according to the present invention, in which, besides the photolabile protective group Y, also a fluorescent radical (in place of R₁) is coupled to the trityl framework.

Figure 5 shows a preferred example of a compound (II),
25 where Q is a group for coupling the photolabile component to the basic trityl framework.

Figure 6 shows a preferred example of a compound (III), where L is a linker group and Q is a group for coupling
30 the photolabile component to the basic trityl framework.